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PRINCIPAL INVESTIGATOR: G. Larry Maxwell, MD

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation
Rockville, MD 20852

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G. Larry Maxwell, M.D.

E-Mail: virginia.vanhorne@na.amedd.army.mil

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: There are significant health-related disparities in outcome among women in the U.S. with different types of gynecologic cancer. HYPOTHESIS: Poor outcome among minorities with gynecologic cancer exists because of biological differences in tumors related to race and ethnicity; cultural, social and psychological barriers to accessing care; less than optimal screening services and prevention strategies, and unequal provision of quality health care and tailored therapeutics. SPECIFIC AIMS: 1: An analysis of the genomic and proteomic expression of gynecologic cancers will be undertaken to determine if there are molecular differences that partially account for the poor outcome among minority patients with gynecologic cancer. This analysis will be expanded in future years to include larger under-served cohorts and comprehensive epidemiological data that will facilitate more detailed genetic and epigenetic analysis. 2: Epidemiological surveys will be used to identify demographic and behavioral differences that lead to poor outcome. We will preliminarily evaluate the use of psychosocial intervention on decreasing morbidity among minorities. 3: Development of Vaccine strategies and specific antibody reagents for the detection of unique targets that are differentially expressed between the African Americans and Caucasians with endometrial cancer. SIGNIFICANCE The incidence, severity, and overall burden of cancer in the U.S. vary by race, ethnicity or other demographic features. This project will focus on identifying the reasons underlying poor outcome among minority groups with gynecologic malignancy so that education, screening, prevention and treatment algorithms can be tailored to high risk populations in an effort to have the greatest impact on reducing morbidity and mortality among the underserved.

15. SUBJECT TERMS
Health disparities, gynecologic cancer, epidemiology, vaccine development

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INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The GCC program project is a collaborative effort between Ohio State University Cancer Center (OSU) and Walter Reed Army Medical Center (WRAMC) with programmatic oversight provided by the Telemedicine and Advanced Technology Research Center of the United States Army Medical Research and Material Command. Improved gynecologic health, particularly among the female active duty population and minority groups, is critically important for the maintenance of readiness among a military force that is composed of an expanding female and minority component. The Gynecologic Cancer Center is a partnership between Walter Reed Army Medical Center and the Department of Defense with Ohio State University Cancer Center to provide optimal unbiased care to gynecologic cancer patients and improve our understanding of racial disparities in outcome that exist for gynecologic cancer. The purpose of the Gynecologic Cancer Center is to identify the etiology of racial disparities in gynecologic cancer incidence and outcome. Tumors and data collected from consenting female military health care beneficiaries are being forwarded to a central data and tissue repository at Walter Reed Army Medical Center. A separate tissue and data acquisition process is being performed for non-DOD beneficiaries at OSU. The information and collected from both types of healthcare systems allows investigators to measure the incidence and prevalence of gynecologic cancer within different racial and ethnic groups and to identify risk factors that may be associated with racial disparities in outcome. Tissues collected within the military and civilian healthcare systems are being analyzed using high throughput molecular analysis (i.e., tissue and oligonucleotide microarray, proteomics, comparative genomic hybridization, etc.) to characterize genetic variation and identify molecular profiles and biomarkers that may be associated with poor outcome in specific racial or ethnic groups. In addition, we are investigating the epidemiologic barriers to care and treatment inequalities that can lead to racial disparities in survival as well as quality of life for minority patients with gynecologic cancer. Using the information obtained from our initial activities, we will implement screening programs for racial and ethnic groups that are at high risk for each type of gynecologic cancer and well as develop novel chemopreventive agents and therapeutics that could be specifically targeted to the risk status of the individual. Currently, the GCC program is operational in a "no cost extension status" and the components of this annual report reflect work that is both ongoing and incomplete in nature.

BODY:

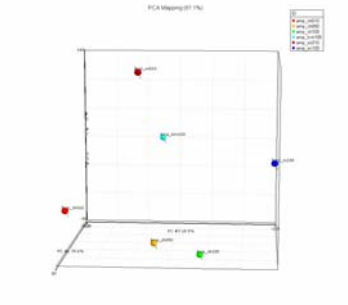
Aim I: Genetics (Morrison)
To identify disparities in genetic and proteomic profiles of minority patients and other groups with health disparities.

Project 1: Oligonucleotide microarray techniques will be used by WRAMC investigators to analyze the genomic expression pattern of African American and Caucasian patients in an effort to identify genetic origins associated with the racial disparity in outcome that is found among African American women with endometrial cancer. These results will be complemented by both methylation specific array and genomic hybridization array of additional endometrial cancer specimens that will be performed at Ohio State University. **(Months 1-12)**. This analysis will be extended to include similar analysis of ovarian and cervix cancer in the future **(Months 8-48)**.

Challenges associated with processing:
During our first year of the program, we analyzed a pilot set of cases using methylation specific and oligonucleotide microarray in a set of endometrial cancers isolated from African Americans versus those obtained from Caucasian women. The sets of cancers were matched for stage, grade and histology. In the first year's analysis, we had chosen to use endometrium that was grossly harvested from the endometrial lining since our pilot oligonucleotdie array data had also been generated from tumors that had been macrodisscted prior to molecular analysis.

- Tissue preparation using Laser Capture Microscopy: During this past year (year 2 of the program), we have initiated laser capture microdissection of tumor and normal control specimens. This method of sample preparation has become the bottle neck for execution of the GCC and other all related projects. In addition, we have faced multiple hurdles that have required resolution before moving ahead with the processing of the case-control sets in a timely manner. As a result, we have obtained a no cost extension for the GCC program to allow us the additional time that is required.
- Time required for case preparation: We currently have two full-time histotechnology technicians that work 50% of their time on the projects related to the GCC program. In addition, a full-time research fellow contributes to the work load requirement; these three personnel are using one laser capture microscope approximately 12 hours a day and 5-6 days a week. Despite the scheduling of shift work that minimizes lost time on the scope, there is still more time required to process samples. Each normal control case requires between 20 and 35 slides to be dissected for normal epithelium to be isolated for DNA and a comparable number for RNA (total of 40-70 slides). Cancer cases require fewer slides (i.e., 15-30) to be dissected but still require significant time for preparation. We have recently received funding to purchase a second laser capture microscope as well as additional personnel (part-time attending staff) who will contribute to the work load requirements in order to get our projects completed in a timely manner.
- Amount and technique used for microarray analysis: An initial analysis was performed to determine whether the amount of material collected for DNA amplification or the method by which the tissue was collected influenced the output from oligonucleotide analysis. We assessed the global expression of each of the sample preparations using PCA and found that they are distinct, suggesting that differences in sample preparation can influence data output. There appeared to be systematic differences in that 10ng and 100ng were more separated than 50ng and 100ng. The distance between 10ng and 100ng is similar both in scrape and chunk indicating that it is probably the effect of dilution. Subsequent discussions with investigators at the Advanced Technology Center at NCI have independently confirmed these findings.

Figure 1: Comparison of methods using principle component analysis



We also found that 100ng and 100ng have similar data values with slight improvement at 100ng over 10ng. The magnitude of this improvement is less than random error level.

Figure 2: Comparison of arrays having maximum and minimum signals using all genes

	# Min signal	# Max Signal
ch010	8940	8263
ch050	6335	7837
ch100	7480	7331
LCM100	14046	12736
sc010	9781	10577
sc100	8092	7927
Total	54674	54671

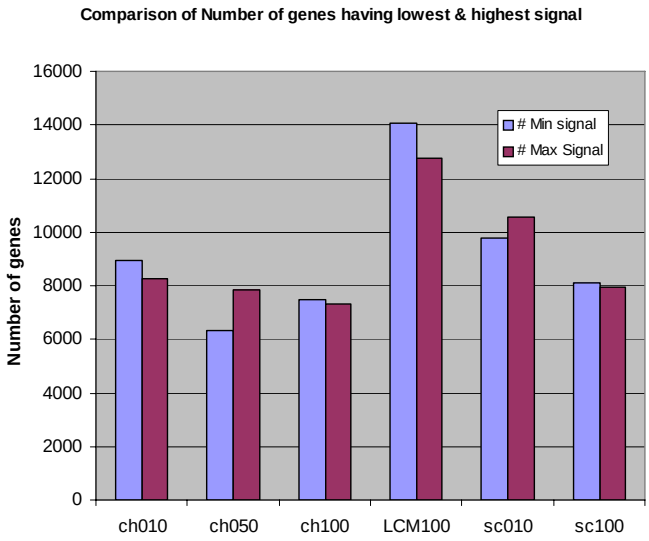


Figure 3: Comparison of signals using data present in all arrays for Scrape 10ug vs 100ug and Chunk 10ug vs scrape 10ug

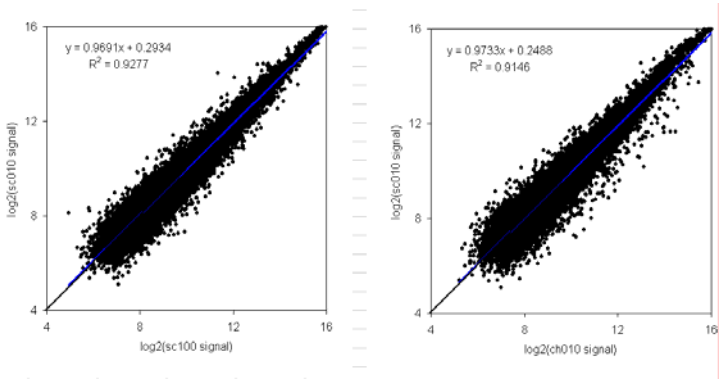
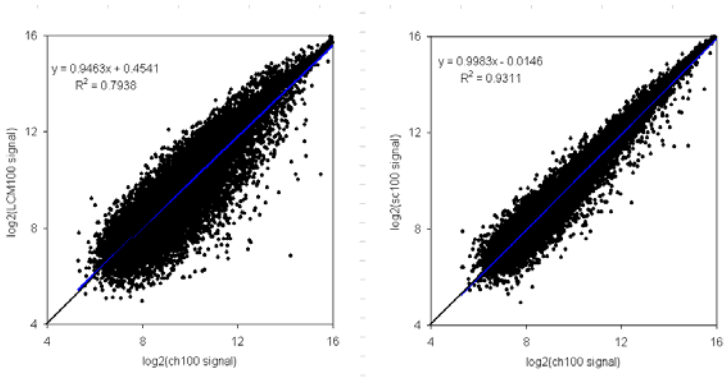


Figure 4: Comparison of signals using data present in all arrays LCM vs Chunk and Scrape vs Chunk



In attempting to obtain some final conclusions in defining the method of processing our samples, we chose to use 50ng for amplification of samples for oligonucleotide analysis. This quantity will be used for all LCM based microarray experiments by our group in an effort to standardize our work in adherence to a quality assurance algorithm. In addition, our group is currently underway with an analysis of 20 samples that were prepared using macrodissection versus laser capture microdissection, both aimed at collection of cancer but with different levels of purity (i.e. gross dissection provides at least 75% cancer cells; LCM provides at least 90% purity). If these two preparation methods prove to be similar across the 20 cases (20 with gross dissection and 20 with LCM), then our group will aim for 75% purity for cancer acquisition using a combination of gross and LCM preparation.

- Cancer sample selection:
    - A matched sample set from Duke University as well as a second sample set from Sloan Kettering have been assimilated to evaluate racial disparities in endometrial cancer. Our group had been asked to write an editorial in response to data reported from Ferguson et al at Sloan Kettering. In this analysis, investigators found an absence of global gene expression differences between African American and Caucasians with endometrial cancer. These findings were similar to those presented by our group at the annual meetings of the Society of Gynecologic Oncology and the American Society of Clinical Oncology. However, when our group removed early stage cases and included only advanced stage cases in the analysis, we found significant differences in global gene expression between African Americans and Caucasians that were confirmed using quantitative PCR. Two genes associated with insulin-like growth factor (IGF1R and IMP-2) were demonstrated to have increased expression among African Americans compared to Caucasians. In addition, the gene PSPH was the most differentially expressed gene in our pilot data as well as the data generated by the Sloan Kettering group. We have therefore hypothesized that there may be specific changes in transcript expression that are commonly found in African Americans with endometrial cancer irrespective of geographical location. There is also the potential for expression differences that might be different between members of a common race group from different areas of the country that are in turn exposed to different epigenetic influences through differences in culture or social environment. We have therefore elected to collaborate with the group from Sloan Kettering in an effort to further evaluate these differences in the original data from our two groups. All samples from both Duke and Sloan Kettering have been collected, embedded and confirmed for adequacy histologically.
    - A third sample set from the Gynecologic Oncology Group was obtained to evaluate using Comparative Genomic Hybridization array (CGH-array). Authorization of sample

acquisition required approvals from the Committee on Experimental Medicine as well as the Uterine Committee of the Gynecologic Oncology Group. Currently, our group is working with bioinformatics and biostatistics experts in the final planning of the experiment. Samples are expected to arrive from GOG in the early fall, facilitating completion of the experiment well in advance of the no cost extension deadline.

- Normal control selection: Many of the microarray experiments to date involving endometrial cancer have focused on comparison between two groups of cancers that are phenotypically different, obviating the need for identification of a suitable control. Comparison of endometrial cancers to a "normal" endometrial specimen is more challenging. Should only epithelial cells or a combination of epithelium and stroma be used? Should postmenopausal or perimenopausal endometrium be used? Does endometrial cancer arise from these differentiated cells or does it derive from a yet to be clarified precursor stem cell? Investigations evaluating multiple controls are forthcoming. Investigations comparing ovarian cancer to normal ovarian tissue have shown previously that the normal control that is selected can strongly influence the genes that are identified as differentially expressed. Although alterations in epithelial-stromal interactions may be important in identifying differentially expressed genes that distinguish cancers, it is also possible that the additional stroma may impede detection of subtle and yet important changes in epithelial gene expression that can be a signature for a particular cancer. In the assessment of whole ovary samples (WO), ovarian surface epithelium (OSE) brushings, OSE exposed to short term culture, and immortalized cell lines, the majority of differentially expressed genes were unique to each cancer versus normal comparison with none of the genes being present on all five gene lists of differentially expressed transcripts. We expect a similar challenge associated with the microarray analysis of endometrial cancer versus normal endometrium. However, these quality control experiments are necessary in the context of microarray studies involving endometrial cancer so that investigative groups can select the more appropriate control in approaching a specific research objective. Our group has used macrodissected for all oligonucleotide array controls to date. Our group is currently underway with quality control experiments designed to evaluate endometrial cancers compared to either a grossly dissected control (stroma plus epithelium) versus a control collected using LCM (only epithelium).

Project 2: Tissue microarray analysis will be used to validate protein expression patterns suggested by the oligonucleotide array, methylation specific array and genomic hybridization array analysis of gynecologic cancers. Construction and/or analysis of tissue microarrays will be performed at OSU and at WRAMC in conjunction with the Armed Forces Institute of Pathology (**Months 1-48**). The focus during **Months 1-12** will be on validating abnormal expression of targeted proteins associated with endometrial cancer that may explain the observed racial disparity in outcome associated with this disease.

Although our group has had some delays in generation of definitive differentially expressed gene lists for racial disparity array projects (aim 1, project 1), we have evaluated the potential racial disparity in expression that might be associated with genes identified in some of our other array projects aimed at identification of molecular profiles associated with poor prognosis tumors. As part of this approach, we have attempted TMA analysis of a panel of over 400 endometrial cancer using an antibody created against ZIC2 (as described in Aim 3). This antibody proved to be nonspecific and our group attempted testing of a second ZIC2 antibody produced as part of Aim 3. The second antibody also proved to be less than robust and no adequate for staining of paraffin samples.

Aim II: Epidemiology and Psychology (Anderson)
To identify social, cultural, demographic and psychological barriers for optimal care of gynecologic cancer among minority patients and other groups with health disparities.

1. Data collected from gynecologic cancer patients at WRAMC, OSU and other collaborating institutions will be used to identify social, environmental, and behavioral risk factors that could partially account for the differences in outcome among minorities with gynecologic cancer **(Months 1-48**).

The Tissue Data Acquisition Activity is a multi-center study involving a number of sites in the Gynecologic Cancer Center Program including WRAMC, Washington Hospital Center, and OSU. Several additional universities (the University of Pittsburgh, and the Windber Research Institute, Duke University Medical Center, and the H. Lee Moffitt Cancer Center & Research Institute) will also participate in this proposed network during subsequent years of this ongoing program aimed at collection of tissue and data from patients diagnosed with gynecologic cancer. Local (DCI) and second level (USAMRMC) IRB approval was obtained in January 2006 to commence the study at WRAMC. Delays in the concurrent activation of support elements of the U.S. Military Cancer Institute's Biospecimen Network subsequently delayed initiation of patient enrollment until this past month. Other concurrent delays in obtaining regulatory approvals for the other sites, both locally and secondarily through Ft Detrick, have subsequently significantly delayed activation of the entire network. Over the summer, Ohio State University, Washington Hospital Center, and the University of Pittsburgh Cancer Institute have been approved (locally/secondarily) with approvals for Duke expected within the next 1-2 months and for the Moffitt Cancer Institute by the end of the year.

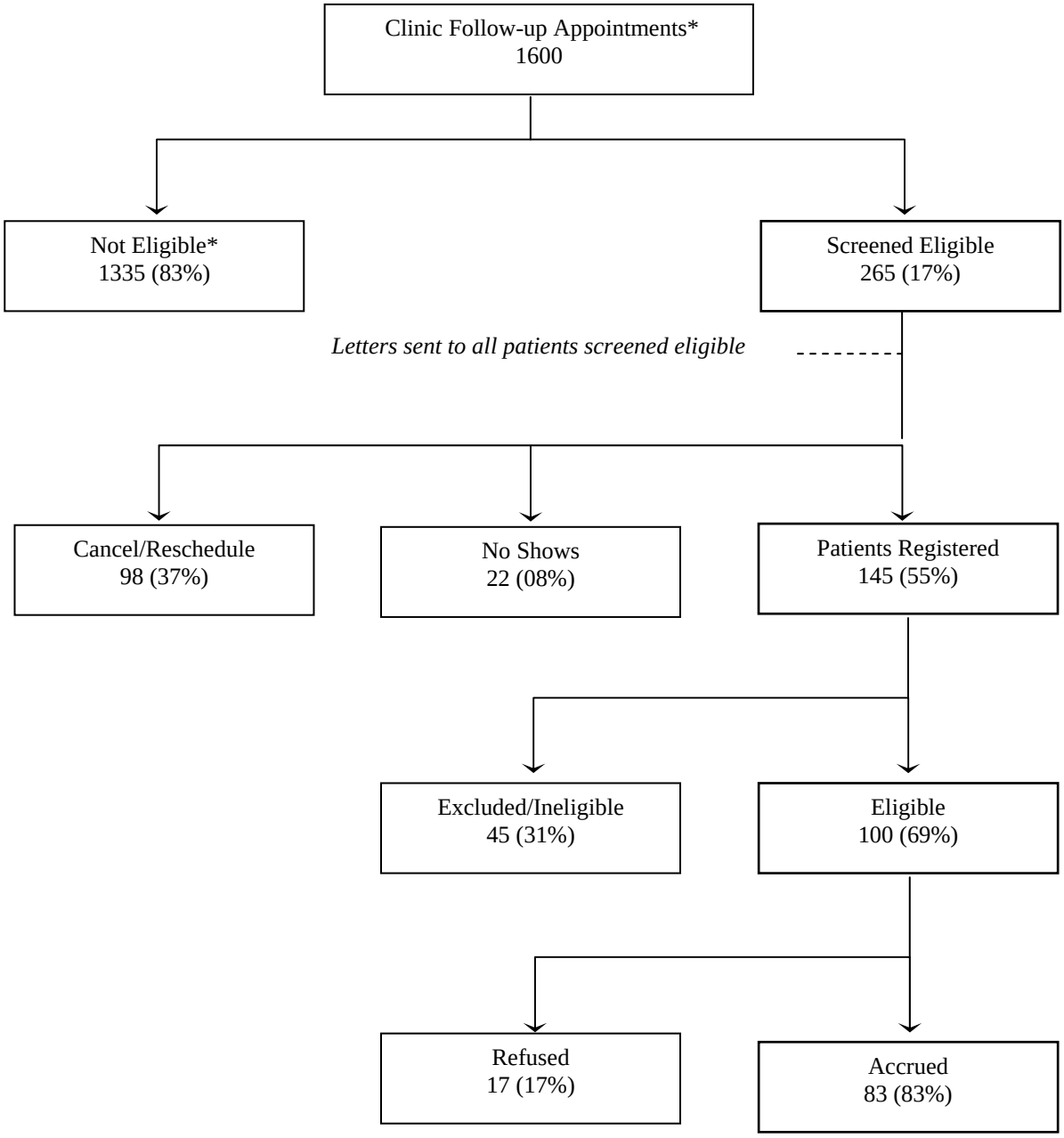
After an extremely prolonged approval of the master protocol involving six primary IRBs and three second level review organizations (Ft Detrick, USUHS, and CIRO) tissue and data collection is underway. During the year that this program project was funded, financial resources were devoted to purchases of equipment, development of the workflow and organizational processes that would occur locally at each institution as well as the algorithm for centralizing data while maintaining a quality assurance method of tissue collection and storage at each institution. Personnel involved in enrollment and data collection were instructed and trained in administration of questionnaires and other data collecting methods.

2. A pilot study focused on endometrial cancer will be performed during **Months 1-12**, since this is the most common gynecologic cancer type and should provide adequate data for preliminary analysis. In this study, we will analyze the association between energy balance and endometrial cancer to determine if differences in diet can partially explain the racial disparity in outcome for endometrial cancer patients. These differences in diet will also be correlated with serum levels of IGF factors and other mediators associated with obesity.

This aim was not initiated because of delays encountered in the activation of the Data and Tissue Acquisition Network that is provided by Core B (Data Acquisition) and Core C (Tissue Banking).

3. A pilot study will test the effectiveness of psychosocial intervention to reduce stress, enhance coping, and prevent sexual functioning morbidity for women with gynecologic cancer. (**Months 1-12**). The preliminary data will be used to implement a larger intervention trial during **months 13-48**.

Figure 5: Flow Diagram for Survivorship Survey at OSU: 7/1/2005-03/07/2006



*** This includes some patients who may have had follow-up appointments between 1/3/2005 & 6/30/2005 as well.**
Excluded/Ineligible: Deemed ineligible based on age, time of diagnosis, or current treatment status.

Table 1: Sociodemographic and disease/treatment characteristics of the total sample accrued from 1/3/05 to 6/30/06 (*N* = 260). OSU accrual is closed as of 3/7/06.

		N = 260	
Variable	%	Mean	SD
Sociodemographic			
Age, years		56.38	12.34
Race, % white	95		
Education, years		14.11	2.76
Family Income, thousand $/year		56.20	42.85
Marital status, % married	62		
Significant other, % yes	70		
Disease group			
Cervical, %	18		
Endometrial, %	51		
Ovarian, %	27		
Vulvar, %	4		
Prognostic			
Stage			
I	60		
II	8		
III	23		
IV	4		
Unstaged	5		
Disease Grade			
1	39		
2	25		
3	25		
4	1		
Not determined	10		
Treatment received			
Surgery, % yes	96		
Radiation therapy, % yes	20		
Chemotherapy, % yes	43		
Time since diagnosis, years		4.19	2.04

Table 2: Means and standard deviations for psychological, quality of life, and sexual characteristic of the total sample accrued from 1/3/05 to 6/30/06 (*N* = 260). OSU accrual is closed as of 3/7/06.

Variable	*N* = 260 Mean	SD	Possible Range
Psychological			
Depression (CESD)	4.30	3.80	0-22
Post-traumatic stress (PCL-C)	26.21	11.97	17-85
Cancer-specific stress (IES)	9.94	10.94	0-75
Body change stress (ITS)	19.08	17.73	0-75
Total mood disruption (POMS)	10.68	7.01	0-28
Total positive meaning (MLS)	13.01	1.94	-3 to 17
Quality of life			
Physical (SF12PCS)	44.27	12.92	
Mental (SF12MCS)	52.71	10.15	
Endometrial-Specific (FACT-En, *n* = 129)	57.14	6.38	0-64
Cervical-Specific (FACT-Cx, *n* = 46)	47.00	6.75	0-60
Ovarian-Specific (FACT-O, *n* = 67)	34.94	5.07	0-48
Vulvar-Specific (FACT-V, *n* = 11)	44.22	8.91	0-60
	Sexually active participants only (N = 128; 49%).		
Sexual functioning and satisfaction			
Female Sexual Functioning Index	24.03	8.21	2-36
Global Satisfaction	4.00	1.89	0-8

- Patients do not exhibit extraordinary levels of mood disruption (POMS) or depressive symptoms (CES-D), with mean scores comparable to available normative data, though approximately 15% exceed cutoff scores for clinically significant depressive symptoms.
- Overall, traumatic stress scores are within range of normative data (PCL-C, IES), though 8 to 9% of patients reported clinically significant symptom levels.
- Body change stress scores (ITS) indicate mildly elevated symptoms.
- In general, patients report high levels of positive meaning derived from their cancer experience (MLS).
- Mental quality of life scores are within range of norms for U.S. adult females, whereas physical quality of life appears to be compromised in this sample (SF12).
- FACT scores are comparable to those of during or within months of active treatment, suggesting that patients quality of life does not improve substantially in the months and years following treatment.
- Sexual functioning scores in the range of patients seeking medical or psychological treatment for sexual difficulties.
- Equivalent for sexual satisfaction is “somewhat inadequate” to “average.”

Correlates of Traumatic Stress among Survivors

The data suggest that long-term gynecologic cancer survivors continue to experience cancer-specific, post-traumatic, and body change traumatic stress symptoms. For example, seven percent of the sample (n = 18) meet the clinical cutoff for post-traumatic stress disorder (PTSD), and an additional 21% (n = 55) meet criteria for the sub-clinical cutoff for post-traumatic stress. Furthermore, 20% of the sample (n = 51) are above the threshold (IES >19) for cancer-specific stress, and 21% (n = 51) report moderate levels of cancer-specific stress (9 < IES <19). Lastly, 19% of the sample (n = 49) report body change stress levels at one-half a standard deviation or above the sample mean (ITS $\geq$ 35).

We hypothesized that gynecologic cancer survivors face lingering physical symptoms resulting from treatment that may be related to such distress. Thus, we explored the association between physical symptoms and the various types of traumatic stress while controlling for other known correlates. The predictor variable, Physical symptoms, was measured using: (1) the Fatigue Symptom Inventory (revised; FSI) – a measure of the frequency and severity of fatigue; (2) Nurse ratings of symptoms and toxicity in the renal/ bladder, gastrointestinal, endocrine and mucosal systems; and (3) the Karnofsky Performance Status nurse rating of functional status. The outcome variables, Traumatic stress, were measured using: (1) the Post-traumatic Stress Check-list-Civilian version (PCL-C); (2) the Impact of Events Scale (IES) – a cancer-specific stress measure; and (3) the Impact of Treatment Scale – a body change stress measure. A series of hierarchical multiple regressions were run including other known correlates of traumatic stress, such as age and comorbid psychopathology. Results suggest that physical symptoms are "predictive" of traumatic, cancer-specific, and body change stress after controlling for age and a history of and/or current depression (see Table 5).

Correlates of Quality of Life among Survivors

In addition, we hypothesized that lingering physical symptoms would also be related to quality of life. We explored this relationship between physical symptoms and measures of quality of life (both global and disease-specific) while controlling for other known correlates. See above for description of predictor variables. The outcome variables, Quality of Life, were measured using: (1 & 2) the physical and mental component summary of the Medical Outcomes Study Quality of Life Short-Form 12 (SF12PCS and SF12MCS respectively) and (3, 4, 5, & 6) the disease-specific quality of life Functional Assessment of Cancer Therapy scales, designed to measure quality of life issues for specific cancers, including endometrial (FACT-En), cervical (FACT-Cx), ovarian (FACT-O), and vulvar (FACT-V). Again, a series of hierarchical multiple regressions were conducted including other known correlates of quality of life, including sociodemographics, disease characteristics, and comorbid psychopathology. Similar to traumatic stress, results suggest that physical symptoms are "predictive" of physical and disease-specific quality of life, after controlling for relevant sociodemographics, disease characteristics, and a history of and/or current depression (see Tables 6 and 7). However, mental quality of life was "predicted" by age and current depression levels, and not by physical symptomatology.

Table 3: Hierarchical Multiple Regressions of Physical Symptoms Associated with Traumatic Stress

Step and Predictor	Statistics by Step		Statistics by Predictor	
	TR^2	R^2 Change	β	*t*
Outcome: Traumatic Stress (sqrtPCLCtotal) (*N* = 260)				
1. Age	.02	.021*	-.069	-1.235
2. History of Depression (dysthymia)	.11	.091***	.191	3.259***
3. Current Depression (CESD)	.20	.088***	.250	4.198***
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.23 4	.033***	.200	3.301***
Outcome: Cancer Specific Stress (sqrtIEStotal) (*N* = 260)				
1. Age	.06	.068***	-.230	-3.844***
2. History of Depression (dysthymia)	.09	.026**	.098	1.570
3. Current Depression (CESD)	.10	.011	.059	.924
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.12	.022*	.164	2.535*
Outcome: Impact of Treatment (sqrtITStotal) (*N* =258)				
1. Age	.11	.112***	-.293	-5.153***
2. History of Depression (dysthymia)	.14	.035***	.094	1.580
3. Current Depression (CESD)	.17	.027***	.107	1.767
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.21 4	.040***	.222	3.586***

* p < .05 ** p < .01
*** p < .001

Table 4: Hierarchical Multiple Regressions of Physical Symptoms Associated with Quality of Life

Step and Predictor	Statistics by Step		Statistics by Predictor	
	TR^2	R^2 Change	β	*t*
Outcome: Physical Component Summary, Quality of Life (*N* = 258)				
1. Age	.04	.048***	-.273	-5.654***
2. History of Depression (dysthymia)	.08	.039**	.031	.596
3. Current Depression (CESD)	.20	.120***	-.098	-1.610
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.45	.243***	-.589	-10.564***
Outcome: Mental Component Summary, Quality of Life (*N* = 258)				
1. Age	.15	.157***	.261	5.442***
2. History of Depression (dysthymia)	.26	.103***	-.101	-1.924+
3. Current Depression (CESD)	.45	.197***	-.510	-8.461***
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.45	.000	-.002	-.029

+ $p = .06$ * $p < .05$ ** $p < .01$ *** $p < .001$

Table 5: Hierarchical Multiple Regressions of Physical Symptoms Associated with Disease-Specific Quality of Life.

Step and Predictor	Statistics by Step		Statistics by Predictor	
	TR^2	R^2 Change	β	*t*
Outcome: Endometrial-Specific Quality of Life (FACT-En) (*n* = 118)				
1. Household Annual Income	.03	.033*	-.036	-.460
2. History of Depression (dysthymia)	.13	.104***	-.087	-1.017
3. Current Depression (CESD)	.28	.146***	-.198	-2.069*
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.44	.165***	-.518	-5.817***
Outcome: Cervical-Specific Quality of Life (FACT-Cx) (*n* = 46)				
1. Employment Status (no vs. yes, full- or part-time)	.20	.202**	.159	1.215
2. Recurrence Status (no vs. yes)	.33	.137**	-.318	-2.890**
3. Current Depression (CESD)	.44	.105**	-.195	-1.397
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.52	.078*	-.379	-2.577*
Outcome: Ovarian-Specific Quality of Life (FACT-O) (*n* =67)				
1. Relationship Status (no partner vs. have partner)	.06	.061*	.210	2.039*
2. History of Depression (dysthymia)	.11	.057*	.029	.238
3. Current Depression (CESD)	.30	.187***	-.374	-3.117**
4. Physical Symptoms (Signs/symptoms, KPS, Fatigue)	.34	.043*	-.260	-2.034*

* $p < .05$ ** $p < .01$ *** $p < .001$

Note: Analyses with Vulvar-Specific Quality of Life were not conducted due to low sample size (*n* = 11).

Aim 3: Treatment (Kaumaya)

Development of Vaccine strategies and specific antibody reagents for the detection of unique targets that are differentially expressed between the African Americans and Caucasians with endometrial cancer.

Project 3 will focus on development of antibodies against targets (identified in Project I) that do not have commercially available antibodies for confirmation of protein expression using tissue microarray (Months 1-12). The project initially will ultimately develop vaccines aimed at decreasing the likelihood of poor outcome among patients with gynecologic cancer that are at risk for a disparity in outcome (Months 16-48). We have expectations of initiating a Phase I study **(Months 18-48)** among endometrial cancer patients following completion of preliminary work.

Development of Antibodies against ZIC2,

O

a gene that has been observed

Prediction of Antigenic Epitopes for Zinc Finger Protein

>gi|22547197|ref|NP_009060.2| zinc finger protein of the cerebellum 2 [Homosapiens]

MLLDAGPQFPAIGVGSFARHHHHSAAAAAAAAAAEMQDRELSLAAAQNGFVDSAAAHMGAFKLNPGAHELSPGQSSAFTSQGPGAYPG
SAAAAAAAAALGPHAAHVGSYSGPPFNSTRDFLFRSRGFGDSAPGGGQHGLFGPGAGGLHHAHSDAQGHLLFPGLPEQHGPHGSQNVL
NGQMRLGLPGEVFGRSEQYRQVASPRTDPYSAAQLHNQYGPMNMNMGMNMAAAAAHHHHHHHHHPGAFFRYMRQQCIKQELICK
WIDPEQLSNPKKSCNKTFSTMHELVTHVSVEHVGGPEQSNHVCFWEECPREGKPFKAKYKLVNHIRVHTGEKPFPCPFPGCGKVFARSE
NLKIHKRTHTGEKPFQCEFEGCDRRFANSSDRKKHMHVHTSDKPYLCKMCDKSYTHPSSLRKHMKVHESSPQGSESSPAASSGYESSTP
PGLVSPSAEPQSSSNLSPAAAAAAAAAAAAAAAVSAVHRGGGSGSGGAGGGSGGGSGSGGGGGGAGGGGGGSSGGGSGTAGGHSGL
SSNFNEWYV

Test	Sequence 1-150	Sequence 151-300	Sequence 301-450	Sequence 451-532
Parker et al hydrophilicity scale	66-91(**2**), 122-135(**3**),	163-173(**2**), 187-206(**4**), 263-277(**2**), 290-299(**3**),	367-382(**3**) 412-436(**3**)	474-523(**4**)
Janin accessibility scale	16-25(**2**), 66-91(**1**), 106-129(**2**).	162-174(**3**), 188-218(**5**), 229-240(**2**), 260-279(**3**),	305-321(**2**) 344-363(**3**), 371-392(**4**) 399-438(**3**)	-----
Bulk hydrophobic scale	61-89(**3**), 106-117(**3**), 124-134(**2**).	182-211(**4**), 262-279(**4**).	305-317(**3**) 371-383(**5**), 412-437(**4**), 441-456(**3**).	477-521(**3**)
Hopp &Woods hydrophilicity scale	32-40(**5**), 114 -131(**3**).	186-205(**3**), 247-275(**4**).	304-320(**5**) 341-385(**5**), 405-428(**3**).	-----
Fraga global scale	30-41(**5**), 114 -131(**3**).	185-205(**3**), 262-274(**4**).	305-320(**5**) 343-384(**6**), 406-427(**3**).	-----
Welling et al antigenicity scale	15-33(**4**), 86-106(**4**), 143-158(**3**).	225-241(**6**).	312-333(**2**) 376-393(**3**),	452-475(**2**).
Hopp acrophilicity scale	60-91(**3**), 105-116(**3**), 124 -145(**4**).	262-274(**2**), 289-299(**3**).	415-455(**2**)	473-525(**6**).
Kyte & Doolittle hydropathy scale	17-23(**4**), 146-153(**4**).	161-176(**4**), 188-218(**5**),	303-319(**4**), 344-367(**4**),	------

		230-240(**6**), 260-280(**4**),	375-390(**3**), 394-425(**2**)	
Novonty large sphere accessibility scale	18-29(**3**), 111-128(**2**).	186-217(**5**), 244-278(**5**),	306-325(**3**), 341-364(**3**),370-385(**6**), 395-425(**5**).	-----
Fauchere & Pliska scale	1-16(**3**), 90-103(**2**) 133-147(**2**).	154-264(**6**), 252-265(**6**),	330-345(**6**), 392-400(**4**),436-444(**3**),	-----
Summary	1-30(**5/10**), 31-45(**2/10**), 61-103(**6/10**), 122-145(**9/10**).	154-173(**4/10**), 188-218(**8/10**), 229-240(**2/10**), 262-280(**9/10**), 281-299(**2/10**).	305-333(**7/10**), 340-367(**4/10**), 365-393(9/**10),** 395-438(**9/10**), 441-456(**1/10**).	452-472(**1/10**), 473-523(**3/10**),

Epitope Selection:

1. **371-391**
2. **392- 435**
3. **120-144**

MVFZFP371-391 Peptide Design and Synthesis

The B-cell epitope (amino acids 371-391 from zinc finger protein of the cerebellum 2, homo spiens) was designed using previously reported strategy (Kaumaya et al) and synthesized co-linearly with a prosmicuous T_H epitope derived from the measles virus fusion protein (amino acids 288-302) with turn sequence GPSL for independent folding of epitopes. All B-cell epi Peptide synthesis was performed on a Milligen/Biosearch 9600 solid-phase peptide synthesizer (Bedford, MA) using Fmoc/t-But chemistry. Preloaded Fmoc-Lys(Boc)-CLEAR ACID resins (0.32 mmol/gm) were used for synthesis (Peptides Interanational, Louisville, KY). Both peptides were cleaved from the resin using the cleavage reagent B (Trifluoroacteic acid:Phenol:Water:Triisopropyl silane 90:4:4:2) and crude peptides were purified as reported earlier (Kaumaya et al, JBC 2004). All peptides were characterized by Matrix Assisted Laser Desorption Ionization mass spectroscopy (MALDI) (Campus Chemical Instrumentation Center, The Ohio State University, Columbus, Ohio). The amino acid sequences and molecular weight information is shown in Table-1.

B-cell epitopes and MVF sequences

Sr. No.	Amino Acid Sequence	Sequence Code	# AA	Mol. Wt.
1	DRRFANSSDRKKHMHVHTSDK Amino Acids 371-391	ZFP371-391	21	2551
2	KLLSLIKGVIVHRLEGVEGPSLDRRFANS-SDRKKHMHVHTSDK	MVFZFP371-391	43	4888

Peptide Immunization and Antibody Purification:

Two NZW rabbits were purchased from Harlan (Indianapolis, IN). Female (outbread) rabbits 6-8 week old, were immunized with peptide construct MVFZFP371-391(1 mg) dissolved in water (500 uL) with 100ug of a muramyl dipeptide adjuvant, nor-MDP (N-acetylglucosamine-3-yl-acetyl-L-alanyl-D-isoglutamine). Peptides were emulsified (50:50) in Montanide ISA 720 vehicle. The same dose of booster injections was administered twice at three and six weeks. Sera was collected by bleed from ear at every week after each immunization for determination of antibody titers. High tittered sera were purified

on a protein A/G-agrose column (Pierce, Rockford, IL) and eluted antibodies were concentrated and exchanged in phosphate-buffered saline using 100-kDa cut-off centrifuge filter units (Millipore, Bedford, MA). The concentration of antibodies was determined by Coomassie plus protein assay reagent kit (Pierce).

Immune response by ELISA:

Antibody titers were determined against B-cell epitope ZFP371-391 and MVFZFP371-391 as previously described (Kaumaya et al 2000 Cancer Research). Ab titers were defined as the reciprocal of the highest serum dilution with an absorbance of 0.2 or greater after subtracting the background. ELISA plot of the crude sera from 2y+3 bleed and purified Ab are shown in Figure-6.

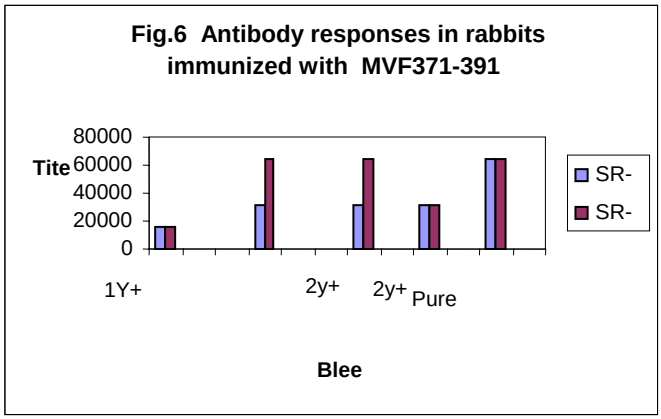


Figure 6. Antibody response against peptides in out bred NZW rabbits. Direct ELISAs were performed on sera from animals immunized with the MVFZFP371-391 construct to determine the immunogenicity. Antibody titers against the corresponding immunogen were defined as the reciprocal of the highest dilution with absorbance $\geq$ 0.2. Designation on the x-axis represents time at which sera was sampled, e.g. 1y+3w corresponds to serum collected three weeks after the first immunization. The sera and the purified Abs were tittered against peptide construct MVFZFP371-391.

KEY RESEARCH ACCOMPLISHMENTS:

- Development of refined algorithms for the processing of laser capture microdissected tissue for microarray analysis.
- Identification of appropriate cancer and control groups for comparison of molecular profiles.
- Completion of all regulatory requirements for multi-institutional genetics and clinical research.
- Creation of antibodies to support analysis.

REPORTABLE OUTCOMES

There have not been any publications or presentations that have yet resulted from this year's work. We plan for presentation of two abstracts related to Aim 2 at this year's annual meeting of the Society of Gynecologic Oncologists. We expect that following completion of the work during the no cost extension, our group will have multiple papers and presentations to provide as deliverables for this program project.

CONCLUSIONS

Our work for this fiscal year is ongoing under a "no cost extension". Final conclusions will be provided with the final report.